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The Family of Human Lymphotropic Retroviruses Called HTLV: HTLV-I in Adult T-cell Leukemia (ATL), HTLV-II in Hairy Cell Leukemias, and HTLV-III in AIDS

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Abstract: The past 5 years have witnessed the beginning of a new era in retrovirology, the era of human retroviruses. Within this short period the causes of two fatal human diseases have been defined as due to human retroviruses. In both instances *in vitro* systems have been developed which mimic the apparent *in vivo* effects of the viruses so that a reasonable amount of information on the involved molecular mechanisms has become available. The future promises much more such information, but what is much less certain is how soon will we learn to correct and/or prevent these diseases.

It is, of course, an honor to give a special lecure at a Princess Takamatsu meeting. It is a special honor to do so on the topic of adult T-cell leukemia (ATL) and human T-lymphotropic virus (HTLV)-I in Japan, where so such of the important ground-breaking research in this field has been conducted, and doubly so to give this lecture in the memory of Henry Kaplan. Dr. Kaplan was the epitome of the basic biomedical scientist who linked his research to clinical applications at every turn. Since his life's work focused on the origins, pathogenesis, and treatment of the lymphoid malignancies and since he believed and sought for a viral cause of these cancers, all of us at this meeting have a special lineage to him. To me he was also a trusted advisor and a much cherised friend who I miss very much. I am grateful to the members of the Scientific Organizing Committee and particularly to Dr. Sugimura, Dr. Sugano, Dr. Miwa, and Dr. Weiss for this opportunity.

My coworkers and collaborators have one-by-one asked me to kindly not speak very much about the molecular biology of HTLV-I, -II, or -III; nor the epidemiology; nor the cell biology; nor . . . , *etc.* This is understandable because they can and will describe this work in more detail and more comprehensively than me. I am then left to describe much about nothing and nothing about much. This doesn't seem too difficult so I will begin. More seriously, my objectives will be to summarize a few of the highlights of the diseases and then the epidemiology of the viruses (HTLV-I, -II, and -III) which cause them, the mechanisms involved, and some of our future aims. I will also give some very brief account of the history which led to the isolation

of these viruses, and I will try to bring out selective interesting questions, problems, and ideas relating to all these topics.

The Viruses: Brief History of the Isolation of HTLV-I and -II

My colleagues and I began looking for retroviruses (leukemia viruses) in humans in 1970 after the discovery by Temin and Baltimore of reverse transcriptase (RT). We were interested in the origin and pathogenesis of human leukemias and sought to carefully test the idea that some might be caused by retroviruses. Their discovery paved the way for the beginning of the biochemical understanding of retrovirus replication, for the making of cDNA probes applicable to all molecular biology, for a new concept on nucleic acid synthesizing enzymes, and to me also a possible powerful new tool for detecting retroviruses in humans by taking advantage of this unique enzyme as a biochemical "footprint" of this kind of virus. We reasoned that if properly assayed, this DNA polymerase might be distinguished from the major normal cellular DNA polymerases and could then be made into a sensitive test for retroviruses. Our idea was that retroviruses, too few to be detected by conventional techniques, might be present in some human leukemias. This idea received considerable resistance mostly because of work on inbred mouse leukemias which indicated that extensive virus replication and viremia preceded leukemia in the viral case of mouse leukemias and apparently also in avian leukemias. Since this amount of virus would easily be detectable by electron microscopy and since so many electron microscopic searches for these viruses were performed in preceding decades, the conclusion was that our attempts would be futile. We and a few other groups felt otherwise, and we saw no *a priori* reason to believe that extensive virus replication was a requirement for a retrovirus-induced leukemia. Therefore, our laboratory and the laboratory of the late Sol Spiegelman, proceeded to develop sensitive and specific assays for RT during the period of 1970 to about 1974 (see refs. *1–3* for a detailed history of this period). These assays are now used routinely in this field, and quite early some important suggestions for the presence of retrovirus information in man was found.

In most cases of ATL, assaying cells for RT would not be sufficient for detecting HTLV-I. Fortunately, in 1972 my coworkers and I also initiated a second approach: an attempt to develop techniques for the long term growth of different kinds of human blood cells. We looked in various biological systems for growth factors. If retroviruses were present in some human leukemias in small amounts, growing the appropriate cells *in vitro* might 1) expand the number of virus particles and 2) possibly enhance the expression of integrated viral genes. Empiric testing of conditioned media from various cell types led to the discovery of T-cell growth factor (TCGF), or interleukin-2 (Il-2) as it is now often called, by my colleagues and myself in the mid 1970s (*4–6*). A "spin-off" of this work was the later finding of the constitutive expression of Il-2 receptors (Il-2R) on the surface of HTLV-I positive primary ATL cells (*7, 8*) and HTLV-I and -II *in vitro* transformed cells (*7–9*). These are subjects of several reports in this meeting. Support for the ideas that viral induced leukemias do occur in the absence of extensive viral replication unexpectedly began to emerge in the early 1970's from the bovine leukemia system. Bovine leukemia virus (BLV) was detected only in *in vitro* growing cells, and as we later learned from the elegant

studies of Arsene Burny and his colleagues the BLV provirus is completely "silent" *in vivo* (see Burny chapter in this volume). As we shall see in this meeting, from the very beginning of the HTLV-I work, BLV was to be the closest animal model for HTLV in man. In time and in turn, HTLV has become a model for BLV again as we will see in this meeting (see reports by Oroszlan and Haseltine in this volume). A point which I think of great interest, but often not discussed, is the fact that BLV causes B-cell lymphomas in cows, but when inoculated into sheep it often produces T-cell malignancies. Recently, Burny and his colleagues reported that when rabbits are treated with BLV they can develop an acquired immunodeficiency syndrome (AIDS)-like immunosupression. Thus, there is evidence that the same virus can in three systems produce disorders of lymphocyte proliferation. These disorders can be hyperproliferation of a T cell, apparent suppression of T cells, and hyperproliferation of B cells. Nature is trying to tell us something with these results. Is it simply the availability of the target cell, *e.g.*, are there more activated T cells in rabbits than cows (T cells must be activated to be infected by a retrovirus)? Feline leukemia virus (FeLV) also offers interesting lessons and questions. It was discovered in the early 1960's by the Jarretts and linked to the cause of cat leukemia by them as well as by Essex and Hardy. It is notable that FeLV causes an AIDS-like disease more frequently than it causes leukemia (see Essex *et al.* in this volume). This time, however, within the same species (cat) the different effects are achieved. Are these different disease manifestations due to variation in the FeLV genome, to host variation, or to variation in other factors, *e.g.*, age of infection, dose of virus, route of infection, presence of co-factors?

Although in 70% of feline leukemias FeLV is found in the tumor, there are extremely interesting findings showing that about 30% of the tumors are FeLV negative. In these latter cases, the normal T cells of the bone marrow are infected. Does this mean FeLV can cause leukemia in an indirect manner and not only by a direct transformation? If so, is this a model for the HTLV-I negative B-cell leukemia in which we have found HTLV-I in normal bone marrow T-cells but not in the B-cell tumor (see Blattner *et al.* in this volume)?

Before proceeding to HTLV-I, something should be said of the first primate leukemia virus to be described, especially since it is appropriate to this meeting. A Japanese-American, T. Kawakami, discovered gibbon ape leukemia virus (GaLV) in the early 1970's. He linked one strain to the cause of a disease very similar to human chronic myelogenous leukemia (as far as I know the only good animal model of this disease) (*10*). He also identified another strain which apparently causes an acute lymphoid leukemia (*11*). Later my colleagues and I found a third strain, which was associated with an acute T-cell leukemia (*12*). Although an extremely difficult model to use, it was nonetheless directly relevant to developments with HTLV in that it supported our belief that some human leukemias might be caused by retroviruses despite older negative results.

The first human retrovirus, HTLV-I, was first isolated in 1978. The discovery was extensively presented at meetings in the U.S. in 1979–1980, and first published in 1980 (*13*). A second independent isolate from my laboratory was reported in 1981 (*14*). During those years some of the viral proteins were purified, hyperimmune sera and monoclonal antibodies were developed, cDNA sequences were obtained, and

evidence for specific antibodies reacting with purified viral proteins was reported (*15–19* and reviewed in *20*). We obtained these isolates from aggressive T-cell malignancies with skin involvement. Our clinicians called these aggressive variants of mycosis fungoides (MF) (*13*) or Sezary syndrome (SS) (*14*). At that time neither they nor much of the world recognized ATL as a specific syndrome, but soon thereafter they and we became aware of it through the work of Takatsuki and his coworkers, Yodoi and Uchiyama (*21, 22*). Therefore, we suspected very early that HTLV-I was the likely cause of ATL in Japan, so we proceeded to seek close collaborations

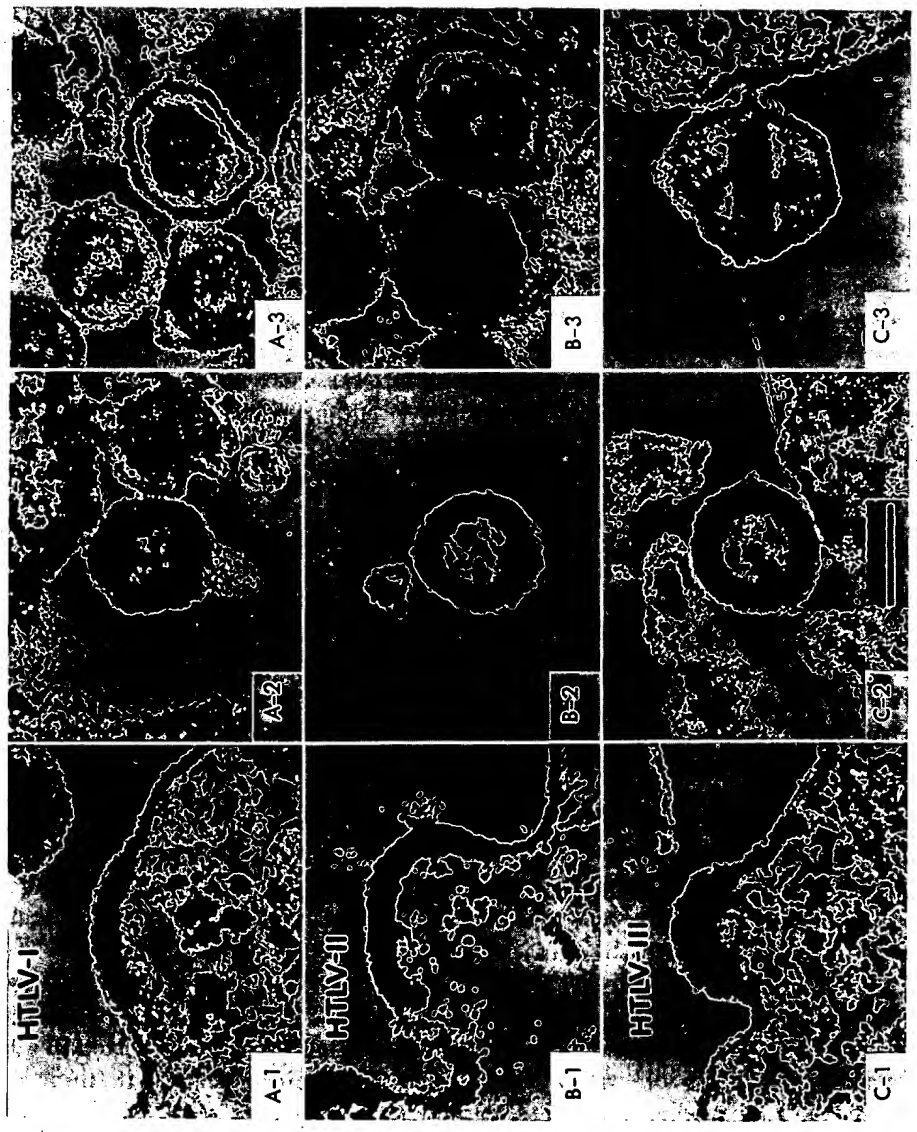

FIG. 1. Electron micrographs of HTLV-I, -II, and -III.

with Japanese colleagues. One collaboration was initiated with Professor Y. Ito of Kyoto and his colleague Dr. Notake, and others were established later with Dr. Nakao of Kobe University and Dr. Aoki and colleagues in Niigata. Coded sera from ATL patients and from healthy donors were sent to us, and we were quickly able to confirm our suspicions. Consequently, we suggested that Professor Ito organize a workshop to discuss these important developments. Our intention was to bring Japanese colleagues into this study widely, and so publication of the Japanese data was delayed. At the March 1981 meeting which Professor Ito organized, we were able to provide reagents and cell lines to Japanese colleagues, and the complete data from my laboratory was presented by myself and three of my coworkers. Dr. Hinuma first presented his EM pictures of Dr. Miyoshi's cell line at this same meeting, but his virus (called ATLV then) was not yet characterized. He also presented some immune fluorescent data with the conclusion that sera of some ATL patients had antibodies reactive with some ATL cell surface antigens ("ATLA") which were then undefined. A summary of this meeting was published in *Cancer Research* (*23*). It should be noted that the development of leukemic cell lines from ATL patients is rare. Routine isolation of HTLV-I still follows the TCGF (Il-2) growth protocol described above, and by this approach there are now more than 50 isolates of HTLV-I in our laboratory and over 100 isolates from various laboratories in the world. Early important confirmations and extensions of this work came from Dr. Haynes and Dr. Bolognesi and colleagues in the U.S. (*24*), Dr. Catovsky and Dr. Greaves and coworkers in England (*25*), and Dr. Vyth-Dreese *et al.* in Holland (*26*, 27). As expected, the "ATLV" in Japan was shown to be the same as HTLV-I (*28*) even to the point of virtual identity by nucleotide sequence analyses.

In 1981 in collaboration with Drs. Saxen and Golde of UCLA we reported the isolation of HTLV-II from a young man (patient MO) with a hairy cell leukemia (*29*). Much has been done on the molecular biology of HTLV-II (*30–34*, and see Chen *et al.* and Shimotohno *et al.* elsewhere in this volume), but we still do not know if it is involved in human disease. The epidemiology of this virus has yet to be elucidated. The morphology of HTLV-I and -II is like that of BLV and differs somewhat from classical type-C retroviruses. The viruses are shown in Fig. 1 and compared to HTLV-III.

The Cell

The vast majority of HTLV-I and HTLV-II infected cells derived from patients or obtained from *in vitro* infection of normal blood cells are mature T cells, usually $T4^+$. Elegant studies described in this book by R. Weiss have shown that T4 is the receptor or part of the receptor for HTLV-III. Therefore, HTLV-III appears highly limited in its host range to T4 cells or to the rare B cells containing T4 antigen. Exceptions to this are cells of the brain (see Wong-Staal *et al.* in this volume). Although *in vitro* studies show the host cell ranges of HTLV-I and -II are much wider (see Weiss *et al.*), and the receptor(s) remain unknown, nonetheless, the disease associated with HTLV-I infection is clearly of the $T4^+$ cell, and the cells transformed *in vitro* by HTLV-I and -II are also predominantly $T4^+$ (*35–37* and see review by Popovic *et al.*, *38*). However, in view of Weiss' indications that other cells might

become infected, perhaps we should give more attention to the possible *in vivo* presence of HTLV-I in other cell types and disease states.

In addition to the T4 marker and relative maturity of the T cells, several years ago we obtained evidence that the HTLV-I positive leukemic T cells and the *in vitro* transformed cells could bind TCGF (Il-2), implying that these cells in contrast to most other human leukemic T-cells have Il-2R on their surface (*7*). Later, this was more accurately studied by binding studies using purified TCGF and the monoclonal antibody called anti-Tac, which is specific for Il-2R. This work has been chiefly done by Waldmann and his colleagues (see Waldmann *et al.* in this volume). We had found that normal T cells do not have Il-2R until they are activated by antigens or phytohemagglutinin (PHA) (*6–8*). Depper *et al.* demonstrated that after activation normal T cells develop about 20,000 Il-2R per cell, whereas ATL cells have many more receptors (*39*). The constitutive expression of Il-2R prompted us to propose that ATL cells may be abnormally growing because of autostimulation, *i.e.*, production of TCGF by tumor cells which respond to their own growth factor due to the simultaneous presence of receptors (*40*). However, this model was disproven when we found little or no detectable TCGF mRNA expressed in most ATL samples or HTLV-I or -II *in vitro* transformed cells (*41*). Yodoi and coworkers discovered a protein released by ATL cells which promotes expression of Il-2R (*42*). This protein which I will call Il-2R activator or ILRA may, in turn, be controlled by long open reading frame (LOR), the protein derived from the coding region of pX. LOR is believed to activate transcription of cell genes critical to T-cell proliferation (see below and chapters by Haseltine and Wong-Staal in this volume). In any case my view is that the expression of Il-2R is probably central to the abnormal T-cell proliferation in this cancer. We still do not know, of course, how a growth factor receptor continually stimulates growth in the absence of its growth factor protein. Other genes possibly relevant to T-cell proliferation are also turned on by HTLV-I or -II transformation. One of these (called HT-3) has been molecularly cloned by Wong-Staal and Manzari in our group, but we do not know its function (*43*).

The Disease

Detailed descriptions of ATL are provided by the clinicians who first distinguished this disease as a specific entity (see chapters by Takatsuki *et al.*, Catovsky *et al.*, Blattner *et al.*, and Gibbs *et al.* in this volume). I will mention only a few features which I think should stimulate laboratory studies.

1. The Lytic lesions of bone and hypercalcemia

These frequent accompaniments of ATL are attributed to activation of osteoclasts, but I am not aware of any detailed studies designed to prove this. Moreover, if due to activated osteoclasts, what activates the osteoclasts? Salahuddin and Markham in my laboratory find that HTLV-I and -II infected T cells release several different lymphokines (*44*). It is often assumed that the infected T cells also make an activator of osteoclasts, but I am not aware of any studies to test this. These lesions, occurring in about half of the cases, are often accompanied by hypercalcemia.

2. The skin lesions

Skin abnormalities in ATL are frequent and of diverse kinds. Little is known about the mechanism of any (exfoliative dermatitis, erythemia, infiltrates, *etc.*). Most intriguing are the frequent infiltrates of the dermis by the infected leukemic T cells. Why do they "home" to the dermis? Is it because of the location of dendritic cells in the dermis? The dendritic cells seem to be macrophage related. If so, are these cells interacting with infected T cells because of some specific receptor interactions? These seem to be testable ideas and areas of research on ATL and HTLV-I which remain open.

3. Short survival

The hallmark of ATL (>85% of cases in the U.S. and Caribbean) is rapid death. How do ATL patients usually die? Hypercalcemia due to osteolytic lesions is one mechanism, and as I said above, we need much more research on this. Perhaps we could prevent some deaths or at least prolong life if we understood the mechanism(s) better. Another frequent cause of rapid death is opportunistic infections (see Broder *et al.* in this volume). The opportunistic infections prompted Essex and coworkers to study infectious disease wards in an endemic area of Japan. He reported a greater incidence of HTLV-I infection in these wards compared to other wards. There are a few alternative interpretations of these results. Essex chose to speculate that this may be due to immune suppressive activity of HTLV-I (*45*). We (particularly Popovic) have collaborated with Fauci and colleagues at NIH, Dupont and co-workers at Sloan-Kettering (*46*), Broder and colleagues at NCI (*47, 48*), Suciu-Foca at Columbia (*49*) to test the *in vitro* effects of HTLV-I and -II on functional normal T cells. In addition, independent studies have been carried out on *in vitro* infected cells by Broder (*50*) and on *in vivo* function of the non-transformed but infected T cells by two Italian visitors, Bonmassar and Macchi, in my laboratory (*51*). The combined results of all of these studies clearly indicate that T cells infected by HTLV-I and -II may or may not be transformed but usually show impairment of immune function and sometimes premature death. In fact, data obtained *in vivo* in chimps support a role of HTLV-I in immunosuppression (Essex, personal communication). Since opportunistic infections are a frequent cause of death we need much more information on the mechanism of HTLV-I induced immunosuppression and how to control it.

4. Other diseases

A major question is whether HTLV-I is involved in diseases other than ATL. As noted earlier, even though the earliest cases in the U.S. were called aggressive variants of MF and SS, they were in fact ATL. Ironically, there is some evidence now to believe that MF and SS are sometimes associated with HTLV-I, *e.g.*, a classical SS case (patient MJ) has been a major source of serum antibody to HTLV-I, and repeated virus isolates have been made from his T cells. Analysis of this virus establishes beyond any doubt that it is HTLV-I (*34*). Moreover, recent more sensitive assays for serum antibodies to HTLV-I have been established in our laboratory by Saxinger (52), and a test of MF patients from Denmark indicate that about 15% are positive (Saxinger *et al.*, submitted). The titer of antibodies are low however,

and viral isolates have not yet been obtained from these patients. We are presently unable to explain these data, but here again is a situation in need of further study.

What about other leukemias and lymphomas? There is strong evidence that HTLV-I is not directly involved in other human leukemias and lymphomas (*53*; and see refs. *20* and *54* for reviews). There is, however, recent exciting results that HTLV-I may be indirectly involved in some B-cell leukemias in HTLV-I endemic areas. If verified this would be consistent with a model proposed several years ago at a Cold Spring Harbor meeting (*1*). Blattner discusses this work in some detail elsewhere in this book.

The Epidemiology

HTLV-I is present only sporadically in most areas of the world, and it is rare in the white population. Known endemic populations include southeastern rural Blacks in the U.S.; parts of Central America; the Caribbean Islands; portions of South America, including regions bordering the Caribbean; southwestern Japan; the Ethiopian Jews of Israel; and European immigrant blacks from the Caribbean or Africa (notably in London and Amsterdam, and probably also Paris). Recent findings of a few ATL cases in southeastern Italy and Sicily (*55*) and low levels of

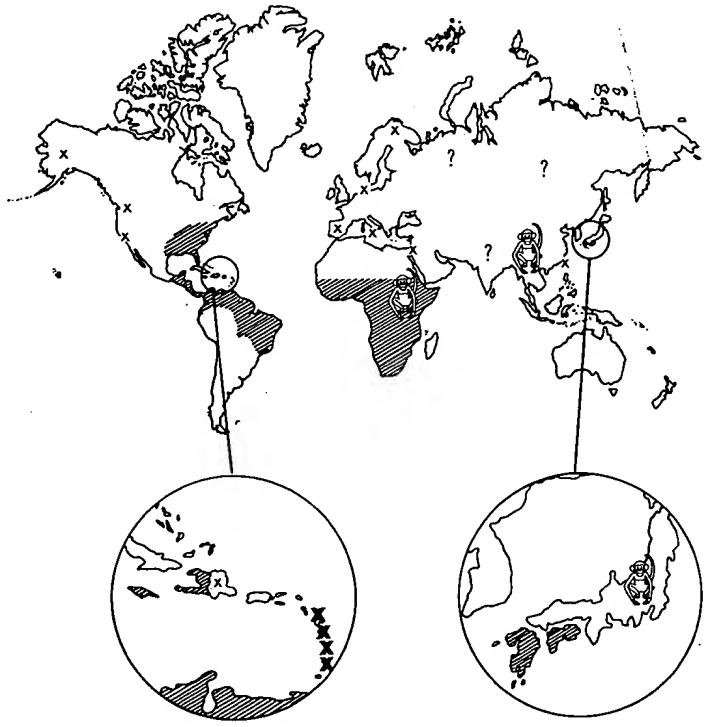

FIG. 2. Areas of the world where HTLV-I infection is endemic and/or where disease clusters have been defined are indicated by X or by shaded areas. HTLV-I is now known to be endemic through much of Africa at a low incidence, but may be more prevalent in Central Africa.

antibody in a small number of people in some parts of Spain (*56*) indicate that these areas may also be regions of HTLV-I presence but at a very low incidence. We found that Africa is the largest land mass of consistent HTLV-I infection (*57*). Studies of de Thé in France and especially Hunsman (see this volume) have reached a similar conclusion. In my view HTLV-I is linked not to similarities in geography or climate, or necessarily to one or a few very specific co-factors as some have often suggested, but rather to a few population types. With so far rare exceptions this virus is predominantly infecting blacks and southern Japanese. Figure 2 summarizes some of the epidemiological points. Moreover, Miyoshi discovered that Japanese macaques are often infected with a virus he originally believed to be HTLV-I (*58*). We (*59*) and Hunsman and his coworkers (*60*) confirmed this result and extended it to several other Old World primates. By analysis of the genome of the virus isolated from some of these animals, we showed that these monkey viruses were very closely related to HTLV-I but could be distinguished from it (*59*). Each species may be infected with its own "HTLV-I variant." This epidemiology seems to tell us a number of things. As I see the results they indicate 1) that HTLV-I is an old primate virus, 2) that it probably originated in Africa, and 3) that it came to the Americas with slave trade and to most other areas of the world by migration of people from Africa. 4) I have previously suggested that HTLV-I probably came to Japan relatively recently (*61*). This suggestion was made because of the identity of HTLV-I in Japan with HTLV-I isolated from the U.S., and because of the prevalence of the original findings showing a striking restriction of the virus to the southwestern part of Japan. We suggested that it may have been brought to Japan by the European (Portuguese?) adventurers who arrived in these very same regions in the 16th Century, were rather restricted to these regions, and apparently brought African servants with them (*61*). This idea has met some resistance, but I am not aware of another explanation of the findings. Support for this notion has recently come from Hino *et al.* (*62*), who in preliminary studies found a rough correlation between ATL and the number of Japanese Christians. Against the idea are some recent results discussed by Hatanaka that the Japanese Ainu in Hokkaido may be an infected group. Some alternative ideas seem to me to have serious flaws, *e.g.*, the concept that HTLV-I is very old in Japan and entered Japanese from monkeys. If this is true why doesn't the virus differ more from HTLV-I of the U.S. or Africa? Also, why aren't the Japanese in other regions of Japan HTLV-I positive? For instance, infected monkeys are found near the Kyoto-Nara region and people from this region are not infected. 5) The main undeniable point that consistently comes out of the epidemiological studies is that HTLV-I is "tightly" controlled, limited to certain very defined regions and within these regions often clustering within families. This argues that transmission is not easy and requires very close contact. Sexual transmission may be one main route of infection. Blood transfusion and *in utero* and/or perinatal infection are other routes of transmission. The point has often been made that it is puzzling that evidence of HTLV-I infection (by serum antibody tests) becomes generally apparent only at the pubertal period, although antibody positive children have been detected (*63*). Perhaps this can be explained by the first sexual contacts. Alternatively, hormonal stimulation of virus replication in a situation in which virus was present at very low levels and/or not previously expressed at all might explain the results. Another and more interesting

idea is one that raises the issue of co-factors. Retroviruses integrate into host cells which synthesize DNA. T cells synthesize DNA only when they are activated. Activation occurs by exposure to antigenic stimulation, *e.g.*, other chronic infections. This might also be relevant to some previous suggestions by epidemiologists in Japan that ATL was correlated with filariasis. Finally, Ito and coworkers have shown that some chemical promoters enhance replication of HTLV-I (*64*). It is possible that sufficient exposure to these or related promoters occurs only by that age.

In Vitro Transformation

I have briefly discussed results showing that HTLV-I and HTLV-II can alter T-cell immune function and speculated that this may be relevant to the development of opportunistic infections in this disease. Another major effect of HTLV-I is, of course, its ability to transform primary human T cells *in vitro*. This is a remarkable phenomenon for several reasons. 1) HTLV-I and -II are the only chronic leukemia viruses which do this. 2) The transformed cells show similarity to HTLV-I positive primary ATL cells. Both are usually T4 positive, Tac antigen positive, show similar morphology, and express some other genes in common. *In vitro* transformation was first demonstrated by Miyoshi (*65*). In his attempt to grow ATL cells he used human fetal cells to support their growth. Apparently and ironically, the ATL cells released virus which transformed his embryo cells. Since the purpose of these experiments was not to transform embryo cells with a virus (at that time unknown), the embryonic cells were not evaluated in advance to see if they were already virus positive. Therefore, in terms of *in vitro* transformation the results were ambiguous. Subsequently, Popovic, Markham, and Salahuddin in my laboratory conducted a series of *in vitro* transformation experiments (*35–37*; and reviewed in ref. *38*). The main conclusions of these studies were: 1) several types of T cells can be transformed but are all intermediate or mature T cells; 2) in most instances the transformed cells are T4; 3) the cells rapidly become oligo or monoclonal; 4) efficient transformation occurs by co-cultivation as first shown by Miyoshi, but more recently we have also been able to transform T cells with extracellular virus (DeRossi *et al.*, submitted). Since this system uses a human carcinogen, HTLV-I, and since the cells transformed are primary human T cells similar to the cells of the disease, I think these systems offer unique opportunities to learn a detailed molecular mechanism of transformation which we can be reasonably confident is relevant to the real situation.

The Mechanism

We have utilized *in vitro* transformed cells to study the molecular mechanisms of *initiation of transformation* and primary ATL cells to learn about mechanisms of *maintenance*. Major classes of human c-*onc* genes were examined in fresh ATL cells and in *in vitro* transformed cells for evidence of rearrangements, amplifications, or abnormally high levels of expression. No gross abnormalities were found. We also found no detectable alterations in the gene for TCGF (Il-2) (Wong-Staal *et al.*, unpublished results), and Greene and Waldmann and coworkers find no evidence of altered Il-2R in the leukemic T cells (see Waldmann *et al.* in this volume.)

We have assayed DNA from fresh ATL cells and the transformed cell lines for HTLV-I specific sequences, utilizing molecularly cloned probes of HTLV-I and the Southern blot technique (*43, 66–68*). The major conclusions from these experiments are listed below.

1) ATL cells contain one (occasionally two and rarely three) proviruses per cell.

2) The provirus in fresh ATL cells is usually complete, but partial proviruses are sometimes found. When found they include pX (see below) and at least one long terminal repeat (LTR).

3) The proviral sequences are monoclonal or oligoclonal, indicating that HTLV-I infected these T-cells *before* they became transformed and not later as a "passenger" virus.

4) The sites of integration differ between different patients and may not even be on the same chromosome.

These findings are reviewed in more detail elsewhere (*20, 68*). Moreover, results from Yoshida's group reached similar conclusions from a larger survey of ATL cases (see Yoshida, Nakahara lecture in this volume for review and references to their work).

At the time of these studies there was one major mechanism suggested for leukemogenesis by a chronic leukemia virus. In avian leukemia the avian leukosis virus LTR induced increased expression of the c-*myc* gene believed to be involved in the leukemic process (*69*). This *cis* mechanism required specific integration sites. The absence of specific integration for the HTLV-I provirus suggested the possibility of a *trans*-mechanism. During this period Seiki *et al.* (*70*) reported the nucleotide sequence of HTLV-I and their discovery of a new set of nucleotide sequences, possibly including one or more genes. They called this region pX. Attention was immediately focused on this segment located at the 3′ end of the HTLV-I genome.

We considered the possibility that pX was a new *onc* gene. However, we found no significant homology to any cellular gene in a survey of many species (*71*), but several results implied that the pX region was essential to initiation of transformation. 1) it was always found integrated in DNA of transformed cells even in those situations where only a partial provirus was found (Wong-Staal *et al.*, unpublished data). 2) It was always expressed in newly transformed cells. 3) The 3′ approximately 1 kb of this 1.6 kb sequence is conserved between HTLV-I and HTLV-II with one long open reading frame (34) termed LOR (72). This is now known to be the coding portion of the pX region. The LOR product is about 42 kdaltons (see Essex and Lee; Haseltine *et al*; Yoshida *et al.*; and the work from Sugimura's group, all described in this volume).

An exciting and important set of experiments with HTLV-I and -II was recently reported, the results of which suggest a new mechanism of leukemogenesis. Sodroski *et al.* from Haseltine's group have shown that HTLV-I and -II LTRs are strong promoters (enhancers) when transfected into cells preinfected with HTLV-I (or II) if those cells are expressing LOR (*73*). Combined with the earlier results described above these findings suggested a *trans*-acting transcriptional activation function by LOR. It is suggested that LOR binds to the HTLV LTR which leads to enhanced transcription of the proviral sequences. How does this lead to T-cell proliferation? We theorize that human DNA contains regulatory sequences similar to the enhancer-

like sequences present in the HTLV LTRs. This leads to enhanced expression of nearby cellular genes which are important to proliferation. But why T cells and not, for example, fibroblasts? Data from Weiss and colleagues have shown that HTLV-I and -II can also enter fibroblasts (*74*). Perhaps the key regulatory elements in T cells which bind LOR have been transposed closer to genes for proliferation during the processes of T-cell receptor gene rearrangement. To summarize, all current data fits with the notion that LOR is critical to the initiation of transformation by HTLV-I and -II. What about maintenance? This so far is a very serious problem. Current evidence indicates that the LOR mRNA is not detected in fresh primary ATL cells (*75*). Although it is possible that this is due to insufficient sensitivity (*e.g.*, short half-life of the mRNA which is expressed at low levels), it is also possible that LOR is not needed to maintain the transformed state. If this is true then we must propose that a second event has taken place; if so it is an event about which we know nothing.

Control of the Disease

In a very rapid period of time more information on the cause and pathogenesis of a human cancer, ATL, has been generated than is available for the cause of the vast majority of human cancers. Nonetheless, our ability to control the disease is negligible. New therapeutic studies are clearly needed. Perhaps more attention should be given to studies designed to augment T-cell function. Probably a focus on the mechanism of constitutive expression of Il-2R will be important; of equal interest will be studies which unambiguously show that constitutive expression of Il-2R is critical to the growth of these T cells. Once understood we may begin to think about rational therapeutic approaches. Waldmann and his colleagues have already taken major steps in developing new treatment. Elsewhere in this book he describes another approach relating to Il-2R. Rather than trying to turn it off, they exploit the Il-2R specificity, and use it to "home" cytotoxic materials to the ATL cell by way of the anti-TAC monoclonal antibody.

Vaccination is, of course, another consideration, and I was a little surprised that a discussion of that approach was not a part of this meeting.

HTLV-III and AIDS

1. The idea

I proposed that AIDS was likely to be caused by a human T-lymphotropic retrovirus in February 1982 at a Cold Spring Harbor Laboratory Conference on AIDS. This idea came out of several considerations which are summarized below.

1) The epidemiological studies were most consistent with an infectious agent. This became extremely likely when the results of blood transfusion associated AIDS were reported.

2) Of various infectious agents, we thought a virus was the best possible explanation because factor VIII could apparently sometimes induce the disease in hemophiliacs, and factor VIII is filtered in a manner that should remove bacteria and fungi. Also, it was clear from the numerous clinical and immunological studies carried out in the U.S. that the T4 cell was the primary target, and I could not think

of a precedent for a bacterium or fungus showing such apparent specificity of infection of a hematopoietic cell: a subset (T4) of a subset (T) of lymphocytes. We could, however, conceive of certain viruses doing this.

3) Because of our experiences with HTLV-I and -II, we thought that among viruses, a human T-lymphotropic retrovirus was the best candidate. This was based on the clear T4 tropism, of HTLV-I, its probable African origin, some immune suppressive activity of HTLV-I and -II *in vitro*, evidence of opportunistic infections in ATL, and the known history of animal retroviruses causing immunosuppressive disease (see Essex *et al.* in this volume).

2. The early problem (a brief history)

We began to test the hypothesis that the primary cause of AIDS was a variant of HTLV-I (or II) or another and distinct human T-lymphotropic retrovirus. In July 1982, our approach was exactly the same as that used for detection, isolation, and characterization of HTLV-I. We obtained primary T cells from peripheral blood, lymph node, or bone marrow of AIDS and "pre-AIDS" patients, and cultured them in TCGF (Il-2) with or without PHA stimulation. By November 1982 transient RT activity was detected and suggestions (by electron microscopy) of a retrovirus different from HTLV-I or -II were found. However, we could not maintain the T-cell growth and continually lost both the virus and the T-cell culture (Fig. 3). We assumed this was due to the poor condition of T cells from AIDS patients. Consequently, the virus was transmitted to cultured peripheral blood T cells from a normal donor. Again, virus was detected but it and the T cells were soon lost (Fig. 3, lower panel). In retrospect, this was due to a cytopathic effect of the virus which we did not adequately appreciate at that time. By February 1983 we had five such "isolates." However, because the virus could not be adequately propagated there was not sufficient material to develop specific reagents to this virus. The only reagents to human retroviruses available in the world at that time were to HTLV-I and -II. Therefore, at that time we could not be convinced that each detection of HTLV-III was indicative of exactly the same virus. We could only conclude that the particles were retroviruses and not HTLV-I or -II. We did not feel these results were sufficiently clear to publish at that time. The group at the Pasteur Institute were stimulated by our hypothesis and according to Chermann of their group they then began to work on the same idea in January 1983 (Chermann, Lerice Conference "International Symposium on Retroviruses and Human Pathology," September 1984), and in May 1983 they reported detection of a retrovirus in T cells of one lymphadenopathy patient utilizing the same TCGF treatment protocol developed for HTLV-I isolation with minor modifications (*76*). They were also able to temporarily transmit this virus to normal T cells. This virus was first called RUB, then LAV, later $IDAV_1$, and then LAV_1. They also suggested it was a human T-lymphotropic retrovirus (*76*). Because of the absence of reagents due to insufficient virus supply they did not report evidence indicating that any two isolates were the same, and although it is clear now that the original and the numerous isolates of LAV_1 and HTLV-III are variants of the same virus, their early work showed only 20 to 30% of sera from AIDS patients contained virus specific antibodies (*77*). Therefore, when we obtained 90% or greater positive antibody results to HTLV-III with AIDS and pre-AIDS sera (*78*, *79*), it was not

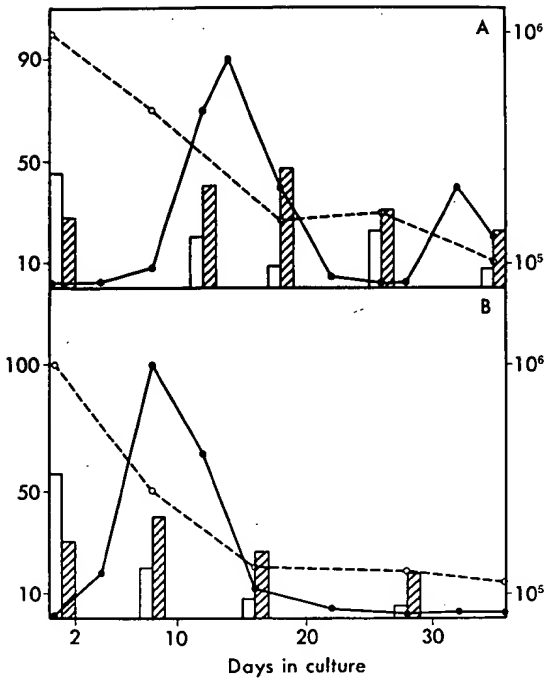

FIG. 3. Release of HTLV-III by cultured cells from a pre-AIDS patient and by infected fresh normal peripheral blood mononuclear cells. Pre-AIDS patient cells were processed, stimulated and cultured as described (*80, 81*) (A). Virus from pre-AIDS patients was used to infect normal human peripheral blood mononuclear cells as described previously (*80, 81*) (B). ○ total viable cell number $\times 10^{-4}$; ● reverse transcriptase activity cpm $\times 10^{-8}$; □ % OKT4/leu3a+ cells; ▨ % OKT8/leu2+ cells.

immediately certain that the viruses were the same. Nonetheless, the Pasteur group first recognized the cytopathic effects of the virus, and was sufficiently convinced of its interest to publish the first identification of the virus.

A major advance occurred in November 1983. When we became aware of the extraordinary cytopathic effect of this virus on T4 cells, we became convinced that we would have to transmit this virus to a permissive cell line to make significant progress. We tested many, but we reasoned that the T4 cell would be the best (perhaps only) target. Since the virus killed normal T4 cells (Fig. 3), we tested leukemic T4 cells available in our laboratory which we knew to be negative for HTLV-I and -II. Elizabeth Read, a technician with us, and Dr. Popovic succeeded in transmitting virus to several lines, but the greatest success by far was with a T4 leukemic cell line we call HT. Clones of this line were then developed; some produce extraordinary amounts of virus with only a slight cell killing effect. The best of these we call clones H9, H4, and H17 (*80*). With the establishment of this permissive cell line, we knew that if the virus was the etiological agent this fact could now be established relatively quickly. We had by that time obtained 48 isolates which were kept in their primary T cells, and these cells were stored still viable for future study (*81*). With massive virus production we could readily obtain the necessary reagents to type each isolate.

This would also give us enough viral proteins to do wide scale epidemiology and should solve the blood bank problem. In addition, it enabled us to make the line available to qualified investigators. Indeed, this is exactly what occurred, and we reported our findings in the spring of 1984 in a series of papers (*78–82*).

An added bonus of the H9 cell line is that after infection some of the T cells become giant and multinucleated and the nuclei take a ring-like form (*80*). Infected cells are often easy to identify, therefore, we named the virus isolated from the original co-cultures with H9 cells HTLV-III because of a previously agreed upon decision at the Cold Spring Harbor meeting on HTLV in 1983 (*83*). The virus was shown to be a human T-cell lymphotropic retrovirus. Some of its proteins are similar in size to HTLV-I and -II. Its RT has the same divalent cation requirements (*80*), and there are common epitopes of the major core protein (Sarngadharan *et al.*, in press) and the envelope between HTLV-III and HTLV-I as detected by human natural antisera (*82*), hyperimmune sera (see Essex in this volume) and by monoclonal antibodies (Mann, unpublished results).

Perhaps most interesting of all, HTLV-III also has a gene analogous to LOR which is also a *trans*-acting transcriptional activator of cellular genes (see Haseltine *et al.* in this volume). This characteristic is of sufficient importance that it has led to discussion about giving a special name to all viruses, BLV, HTLV-I, HTLV-II, HTLV-III, and visna, which possess it: *i.e.*, type T retroviruses (T for *trans*-acting). Having said this it should be emphasized that there are major differences in the genomes of the several HTLV subtypes, *i.e.*, HTLV-III is not simply a variant of HTLV-I or -II (see Wong-Staal *et al.* in this volume).

3. Experimental biological effects of HTLV-III

We now know of at least three laboratory results of HTLV-III infection: 1) T4 cell killing; 2) induction of multinucleated giant cells with nuclei arranged in a circular pattern; 3) *in vivo* infection of chimpanzees resulting in a diminished T4/T8 ratio and the development of lymphadenopathy and persistent antibody (over 60 weeks) (*84*).

Establishing the Etiological Link of HTLV-III to AIDS

1) The ability to kill human T4 cells, 2) the capacity to produce lymphadenopathy in chimps, 3) the apparent recent entry of the virus into industrialized societies, and 4) the animal models of retrovirus induced T-cell immunodeficiency are obviously consistent with HTLV-III being the cause of AIDS. I think two additional results make this conclusive (see below).

1. The reproducible isolation of HTLV-III from AIDS, pre-AIDS, and appropriate risk groups

We originally reported 48 isolates of HTLV-III from T cells of AIDS, pre-AIDS, and risk groups (*81*). We have now over 100 isolates of HTLV-III. A few were selected for large scale production and form the prototypes of these viruses in our laboratory. By making repeated attempts and by the use of hydroxycorticosteroids, Markham and Salahuddin in our laboratory has increased the incidence of virus iso-

lation to more than 90% in pre-AIDS and more than 50% in AIDS (Salahuddin *et al.*, in press). Culture of peripheral blood T cells from people belonging to known risk groups (*i.e.*, intravenous (i.v.) drug users, Haitians, prostitutes, and promiscuous homosexuals) yielded virus in 30% of all attempts, whereas culturing these cells from over 150 normal healthy heterosexuals did not yield detectable virus in any. Table 1 lists 95 of these isolates of HTLV-III.

Originally, we isolated HTLV-III from T cells obtained from blood, bone marrow, lymph nodes, and spleen (*81*). More recently we have isolated it from plasma as infectious extracellular particles (Salahuddin *et al.*, submitted). This is in contrast to HTLV-I which to my knowledge has never been isolated from cell free fluid. These findings are consistent with the association of AIDS with whole blood or factor VIII transfusions. Considerable epidemiological evidence suggests that AIDS is linked to sexual practices. However, virus had not been identified in semen until

TABLE 1. Summary of the Isolation of HTLV-III from AIDS and ARC Patients, and Clinically Normal Donors at Risk for AIDS

Description of patients and donors[a]	Diagnosis	Number of HTLV-III isolates[b]
Homosexual males[c]	AIDS	36
	ARC	21
	Clinically normal[d]	14
Non-homosexual males and females		
Intravenous drug users	ARC	2
Hemophiliacs	AIDS	4
	ARC	1
Other transfusion recipients	AIDS	1
	ARC	1
Juveniles	AIDS	3
	ARC	1
Mothers of juvenile AIDS[e]	AIDS	1
	ARC	1
	Clinically normal	2
Promiscuous males	AIDS	2
	ARC	6
Spouses of AIDS and ARC patients	ARC	1
	Clinically normal	3
Prostitutes	ARC	1
Random donors	Clinically normal	0

[a] Peripheral blood leukocytes, bone marrow, or lymph nodes, were collected, processed and introduced into *in vitro* culture as previously described (*80, 81*). Saliva (*86*) and plasma (Markham *et al.*, in preparation) were processed and used to transmit virus directly to fresh or established cell lines in some instances. All but five of the AIDS/ARC patients and donors at risk for AIDS reported here were seropositive for antibody to HTLV-III (78). [b] Isolation of virus is as described (*80, 81*). The overall incidence (number of virus isolates/number of patients tested) was 50% (44/88) for AIDS patients, 85% (34/40) for ARC patients, 50% (12/24) for clinically normal promiscuous healthy homosexual males, 14% (5/35) for clinically normal, non-promiscuous, healthy homosexual males, and other donors at high risk for AIDS, and 0% (0/125) for random, seronegative, donors. [c] These include four male, Haitian immigrants that may or may not be homosexuals. [d] These include one clinically normal homosexual donor who donated plasma to at least three juveniles who subsequently developed immune deficiency. [e] Additional risk factors for AIDS identified in two mothers, were heterosexual promiscuity (prostitution) and i.v. drug abuse.

recently. Zagury and coworkers of the University of Paris grew single T cells (with TCGF) from semen of AIDS patients and HTLV-III was found in these cells (*85*). Simultaneously, Hirsch and his coworkers isolated HTLV-III from semen (*87*). Somewhat unexpectedly, in collaboration with Groopman, we were also able to isolate HTLV-III from saliva of pre-AIDS patients and from several healthy homosexuals (*86*). Epidemiological studies indicate that HTLV-III is not casually transmitted by saliva (*e.g.*, sneeze, cough, *etc.*) but I know of no data to exclude heavy salivary exchange as a mode of transmission. The isolation of infectious virus from saliva is at minimum a clear signal that we need to pay more attention to this source as a possible route of transmission under some circumstances.

2. Serological data linking HTLV-III to the cause of AIDS

Sarngadharan describes some of these results in detail, including the methodological approaches elsewhere in this book. These studies were done by him, Saxinger, Robert-Guroff or Schupbach in our group, in collaboration with several groups. Groopman (Boston), Safai, Gold, and Armstrong (Sloan-Kettering); Haynes (Duke), White (University of North Carolina), Redfield (Walter Reed Army Hospital), Steigbigel and coworkers (Montefiore Medical Center), Fauci and Lane (NIH), Broder, Blattner, Goedert, and Biggar (NCI, NIH), Curran and Jaffe (CDC, Atlanta) made substantial contributions. In addition, numerous collaborators from different countries including Japan took part in some studies. Some of the major conclusions are listed below.

1) HTLV-III specific antibodies react early in disease with both the major core protein (p24) as well as with larger proteins which we believe to be envelope proteins. However, late in AIDS sometimes only antibody to the envelope proteins are found. Since Montagnier and colleagues have primarily used p24 (p25) as the antigen for screening, this could be another reason why they originally reported much lower rates of positive results in sera of AIDS patients to LAV.

2) More than 90% of AIDS sera and about 90% of sera from people with so called AIDS related complex (ARC) or "pre-AIDS" react with HTLV-III proteins, whereas less than 0.2% of normal heterosexuals screened in the U.S. in 1983 or early 1984 were positive.

3) All AIDS risk groups show a sizable fraction of positive sera. This includes homosexuals, Haitians, Africans, i.v. drug users, and recipients of frequent blood transfusions, plasma or factor VIII (*78, 79, 88–90*).

4) New risk groups are developing in the U.S. and now appear to include promiscuous heterosexuals (*91*; Harris *et al.*, in preparation) including prostitutes and infants born of prostitutes, Haitians, and i.v. drug using mothers (*92*; Robert-Guroff *et al.*, unpublished data).

5) The results of blood transfusion associated AIDS alone strongly indicate HTLV-III is the cause of AIDS (Jaffe *et al.*, in press).

6) People who are immune suppressed for other reasons only very rarely are positive for HTLV-III (*78*). This is, of course, consistent with HTLV-III being the cause of AIDS rather than a secondary opportunistic infection in an already immune compromised individual. I would not have expected the complete lack of opportunistic infection by HTLV-III in people immune suppressed by various factors.

An intriguing aspect about this is that such people may ironically be partially protected against HTLV-III infection. Successful integration of the retroviral genes may require cell proliferation. T cells proliferate when activated but immunosuppressed people may have very few cells which are activated or can be activated.

7) The results of prospective epidemiological studies also indicate HTLV-III is the cause of AIDS (*93*).

8) Results of numerous collaborative studies show that HTLV-III is a new virus infection now present throughout Europe (*88*, *94–96*; Aiuti *et al.*, submitted), U.S., Australia (*97*), and, of course, Africa (Clumeck *et al.*, in preparation).

The origin of HTLV-III cannot be precisely defined nor can that of the time and place of where and when the AIDS epidemic began. However, recently Saxinger in our laboratory collaborating with other investigators who collected sera in Uganda in 1972–1973 as part of a Burkitt lymphoma study, found that about 65% of rural, poor, Ugandan children from the West Nile Bank region had low levels of antibodies to HTLV-III (*98*). Since the AIDS epidemic became apparent some years later in Africa this may mean that the virus changed since 1972 and became pathogenic. Alternatively, these children may represent a resistant population. Infant mortality is high in this region from infections. It is possible that this is in part due to endemic HTLV-III infection. Some children may be genetically able to resist serious disease from infection. For example, as discussed earlier and reported in detail by Weiss at this meeting, he and his colleagues discovered that T4 is part of the HTLV-III receptor. He proposed that some variation in susceptibility might develop from polymorphism of T4, a phenomenon already known to exist in some black Africans (Rietmuller, personal communication). Thus, infection may be quite limited in some who are then able to survive the infection and exhibit low level antibody titers.

The above studies are, of course, consistent with the epidemiological suggestions that AIDS began in Africa. From what we have learned we suspect it originated in Central Africa. How did it get to Haiti, the U.S., Europe and elsewhere? There is *no answer to this important question*, but some interesting points to consider are 1) contacts between Zaire and Haiti, 2) contacts between American homosexuals and Haitians, 3) African contacts directly to Europe, and 4) the Cuban soldiers foray into Angola and neighboring regions.

3. Co-factors

Are other factors needed to cause AIDS? When too many co-factors are suggested as etiologically involved in a disease we are usually very ignorant of the primary cause. In our view, if sufficient HTLV-III enters the blood of an individual, it is likely the individual will become infected. The percentage of infected people who develop disease is unknown but Blattner, Goedert, and their coworkrs have estimated that about 6% per year develop disease. This is, of course, a very sizable number. It is probable that infections with other agents increase the number of T cells infected by HTLV-III by activating the T cells so that DNA synthesis is initiated. In this sense chronic infections may be co-factors.

Molecular Biology of HTLV-III

Wong-Staal and her coworkers (Hahn, Arya, and Shaw) in our group have recently published on the first molecular clones of HTLV-III from several isolates (*99*, *100*), and the full genome has been sequenced in collaboration with a few other groups (Haseltine and coworkers; Chang; Papas and coworkers; and especially Pearson and his coworkers from the Life Science Devision of the DuPont Company (*101*)). Many interesting points have come from these studies which are described elsewhere in this book by Wong-Staal and also by Haseltine. I will reiterate only three. First, the genome is polymorphic (in contrast to HTLV-I); second, the data indicate that HTLV-III does not directly cause Kaposi's sarcoma; and third, the genome contains extra genes and one of them must be involved in the transactivation phenomenon.

Control of HTLV-III

Broder addresses this question in detail elsewhere in this book. Inhibiting virus replication, killing reservoir cells, and replacing lost T cells by transplantation and possibly by Il-2 therapy are perhaps the best ideas for treating an infected and already sick person. Vaccines are being actively pursued by different approaches in several laboratories. Two considerations have made us less optimistic about vaccines: some genomic polymorphism of different HTLV-III isolates (*102*) and the evidence that people may not make very high titer of neutralizing antibodies to HTLV-III (Weiss elsewhere in this volume).

A summary of some of the features of HTLV-III in common with HTLV-I are listed in Table 2. However, it is emphasized that there is little overall genomic homology.

TABLE 2. Relatedness of HTLV-III to HTLV-I and HTLV-II

	Subgroup of HTLV		
	I	II	III
1. General infectivity	Lym	Lym	Lym
2. Particular tropism	T4	T4	T4
3. Diameter	110–140 nm	110–140 nm	110–140 nm
4. RT size	~100K	~100K	~100K
5. RT divalent cation	Mg^{2+}	Mg^{2+}	Mg^{2+}
6. Major core	p24	p24	p24
7. Common envelope epitope	+	+	+
8. Common p24 epitope	+	+	+
9. Close homology to other retroviruses except PTLV[a]	0	0	0
10. pX (*tat* gene)	+	+	+
11. Produces giant multinucleated cells	+	+	+
12. African origin	Likely	?	Likely
13. Transactivation	+	+	+

[a] PTLV is also referred to a STLV (simian T-lymphotropic retroviruses).

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